New record of the Japanese Seahorse *Hippocampus mohnikei* Bleeker, 1853 (Syngnathiformes: Syngnathidae) in Hong Kong waters

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Abstract

A new record of *Hippocampus mohnikei* Bleeker, 1853 was confirmed in Hong Kong through morphological and molecular identification. It is highly possible that there is an established population of *H. mohnikei* in Hong Kong due to continuous sightings by citizen scientists in the past nine years. The record is significant from a biogeographical perspective as it connects the distributions of known populations in northern China and Japan to those spanning Thailand to India. This further affirms the status of Hong Kong as a hotspot for seahorse biodiversity and conservation in China.

Keywords

Distribution expansion, South China Sea, species distribution; subtropical region

Introduction

Hong Kong, on the southern coast of China, is one of the most densely populated major cities in the world. Despite its small area (approximately 0.03% of China's total marine area) and proximity to urban areas, Hong Kong is a region of important marine biodiversity, as it is home to ~26% of the total marine species recorded in China (Ng et al. 2017).

Hippocampus Rafinesque, 1810 is a genus of fish commonly referred to as seahorses and belongs to the family Syngnathidae. It comprises 46 extant valid species (Lourie et al. 2016; Zhang et al. 2016; Han et al. 2017; Short et al. 2018, 2020). Historically, however, taxonomic confusion and the lack of studies have impeded conservation for these rare marine fish around the world (Lourie

2004), including China. There are six seahorse species that have been officially recorded in China: *H. kelloggi* Jordan & Snyder, 1901, *H. kuda* Bleeker, 1852, *H. mohnikei* Bleeker, 1853, *H. spinosissimus* Weber, 1913, *H. trimaculatus* Leach, 1814, and *H. casscsio* Zhang, Qin, Wang & Lin, 2016 (Zhang et al. 2016; Zhang and Vincent 2017). Except for *H. casscsio* which is Data Deficient, the other five species are Vulnerable, as currently listed in the IUCN Red List of Threatened Species (IUCN 2022). Besides, all of them are also listed in Appendix II of the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora. Three of the six species (*H. kelloggi*, *H. kuda*, and *H. trimaculatus*) have been officially recorded in Hong Kong waters before this survey (Astudillo et al. 2022).

Seahorses have been commercially sought after for trade as ingredient in traditional Chinese medicine, souvenirs, and live aquarium pets in recent years (Lin et al. 2008; Koldewey and Martin-Smith 2010). Demersal non-selective fishing (e.g., bottom trawling), pollution, and other anthropogenic pressures have imposed great threats upon global seahorse populations, especially in China's seas (Zhang and Vincent 2019). This is concerning given the vulnerable functional and life history traits (slow-moving, highly site-specific, and low fecundity) of seahorses (Aylesworth et al. 2016).

The Japanese Seahorse, *H. mohnikei*, has been officially recorded in Japan (Kanou and Kohno 2001; Suda et al. 2004; Otsuka et al. 2009; Yagi et al. 2011), northeastern China (Zhang et al. 2014), Vietnam (Aylesworth et al. 2016), Malaysia (Aylesworth et al. 2016), Singapore (Lim 2013), South Korea (Kwak et al. 2008; Choi et al. 2012), Thailand (Aylesworth et al. 2016), Cambodia (Aylesworth et al. 2016), and India (Thangaraj and Lipton 2007; Lipton and Thangaraj 2013; Sanaye et al. 2019). These records demonstrate that *H. mohnikei* generally prefers shallow waters (<10 m deep) in various habitats including sheltered bays, mangroves, seagrass beds, estuaries, and sandy substrates. Moreover, one of us (Yiu SKF) has encountered an individual of *H. mohnikei* in eastern waters of Hong Kong in 2016 while recreational diving. It was reported to the Ocean Park Conservation Foundation, Hong Kong and published in a e-newsletter (OPCFHK 2017). However, there was no molecular data to support its identification. The sighting records from recreational divers showcase the importance of citizen scientists, who, although untrained and from a wide range of educational backgrounds, can contribute to scientific studies (Silvertown 2009). Before this study, the lack of *H. mohnikei* records in southern China suggested that there are two large populations occurring in east and southeast Asia, respectively. In addition to the new record of *H. mohnikei* described in detail here, we have also collated and confirmed sightings in Hong Kong over the past nine years to confirm through morphological and molecular methods that a population exists in the southern China region. We believe this also confirms the continuity of the species distribution throughout east and southeast Asia.

Methods

Data collection. A Japanese seahorse was observed underwater in Pak Lap Tsai on 31 July 2018. Photos of the individual were taken in situ during underwater visual census surveys in accordance with protocol and calibration described in Côté and Perrow (2006) and Tam and Ang (2009). The individual in our photographic records was identified according to morphological identifiers (i.e., double cheek spines, shorter snout, and long tail in proportion to body) outlined by Lourie et al. (2004) and further verified by one of us (Zhang X) from Project Seahorse. In order to include other informal sightings of *H. mohnikei* in Hong Kong, data were collated from Global Biodiversity Information Faculty (GBIF), Oceanic Biodiversity Information System (OBIS), FishNet, FishBase, and iNaturalist, and we reached out to local recreational divers. The identification of all photo records collected were confirmed using same protocol mentioned above. To obtain the molecular data with minimal impact on the individual seahorse and the seahorse population, non-detrimental partial fin-clippings of two local specimens were collected for DNA extraction and PCR in 2019. The seahorses were released alive after sampling.

Sampling, DNA extraction and polymerase chain reaction (PCR). A tiny portion of fin tissues of two local specimens from waters off Lantau Island were collected in 2019, following the protocol in Woodall et al. (2012). The surface of each tissue sample was decontaminated using 10% bleach solution followed by adequate washing with sterile MilliQ water. DNA extraction was performed using 10% Chelex®100 (Bio-Rad) with 1 h digestion using 2 µl proteinase K (TaKaRa).

A 680-bp fragment from the region of the mitochondrial *COI* gene was amplified using the primers VF1_tl: 5′ - TGT AAA ACG ACG GCC AGT TCT CAA CCA ACC ACA AAG ACA TTG G - 3′ (43 bp) VR1_tl: 5′ - CAG GAA ACA GCT ATG ACT AGA CTT CTG GGT GGC CAA AGA ATC A - 3′ (43 bp). PCR was performed in 25 µl of reaction mixture containing 18 µl sterile MilliQ water, 2.5 µl 10 × PCR buffer (Mg2+ plus, TaKaRa), 0.15 µl of each primer (10 µM), 2 µl dNTP (2.5 mM), 0.2 µl Ex Taq DNA polymerase (TaKaRa), and 2 µl DNA template. Thermal cycling was performed on a C1000 Thermal Cycler (Bio-Rad) at 95 °C for 2 min, followed by 1 min at 94 °C, 45 s at 51°C, 45 s at 72 °C, and 39 cycles of 94 °C for 1 min, 51 °C for 90 s, 72 °C for 45 s, with a final extension at 72°C for 20 min. The PCR products were visualized on a 1.5% agarose gel. The PCR products were then submitted to BGI HK for purification and sequencing. The sequence data had been deposited in NCBI's GenBank (Table 1).

Phylogenetic analysis. Alignments of the sequences was conducted and trimmed manually to 611 bp using

Table 1. GenBank accession numbers of the sequences used in phylogeny reconstruction and species delimitation retrieved from NCBI.

Species	Accession no.
Hong Kong specimen 1	MW081994
Hong Kong specimen 2	MW081995
Hippocampus barbouri	MH732962
Hippocampus barbouri	MH732963
Hippocampus kelloggi	KT823928
Hippocampus kelloggi	KT823929
Hippocampus kuda	KJ883549
Hippocampus kuda	KJ883554
Hippocampus mohnikei	MT558638
Hippocampus mohnikei	MT558639
Hippocampus spinosissimus	KJ883573
Hippocampus spinosissimus	KJ883574
Hippocampus trimaculatus	AB983270
Hippocampus trimaculatus	AB983274

MEGA v. 7. The trimmed sequences were imported to the website version of IQ-Tree (Nguyen et al. 2015) for maximum-likelihood tree reconstruction with 1,000 ultrafast bootstrap pseudoreplicates (Hoang et al. 2017). The phylogenetic trees were visualized and edited using FigTree v. 1.4.4. Pairwise genetic distance was estimated using MEGA 7 based on the *p*-distance method using the bootstrap method with 10,000 pseudoreplicates for variance estimation. Rates among sites were gamma distributed with invariant sites (G+I) and the gamma parameter was set to four.

Results

Order Syngnathiformes
Family Syngnathidae Bonaparte, 1831
Genus *Hippocampus* Rafinesque, 1810

Hippocampus mohnikei Bleeker, 1853
Figure 1

New record (Fig. 2). CHINA – **Hong Kong** • Sai Kung; Pak Lap Tsai; 22°21′12″N, 114°22′04″E; 7m depth; 31.VII. 2018.

Habitat. The site is composed of large boulders, sand, dead coral, and patchily distributed colonies of table corals (*Acropora* spp.) and other encrusting scleractinian corals.
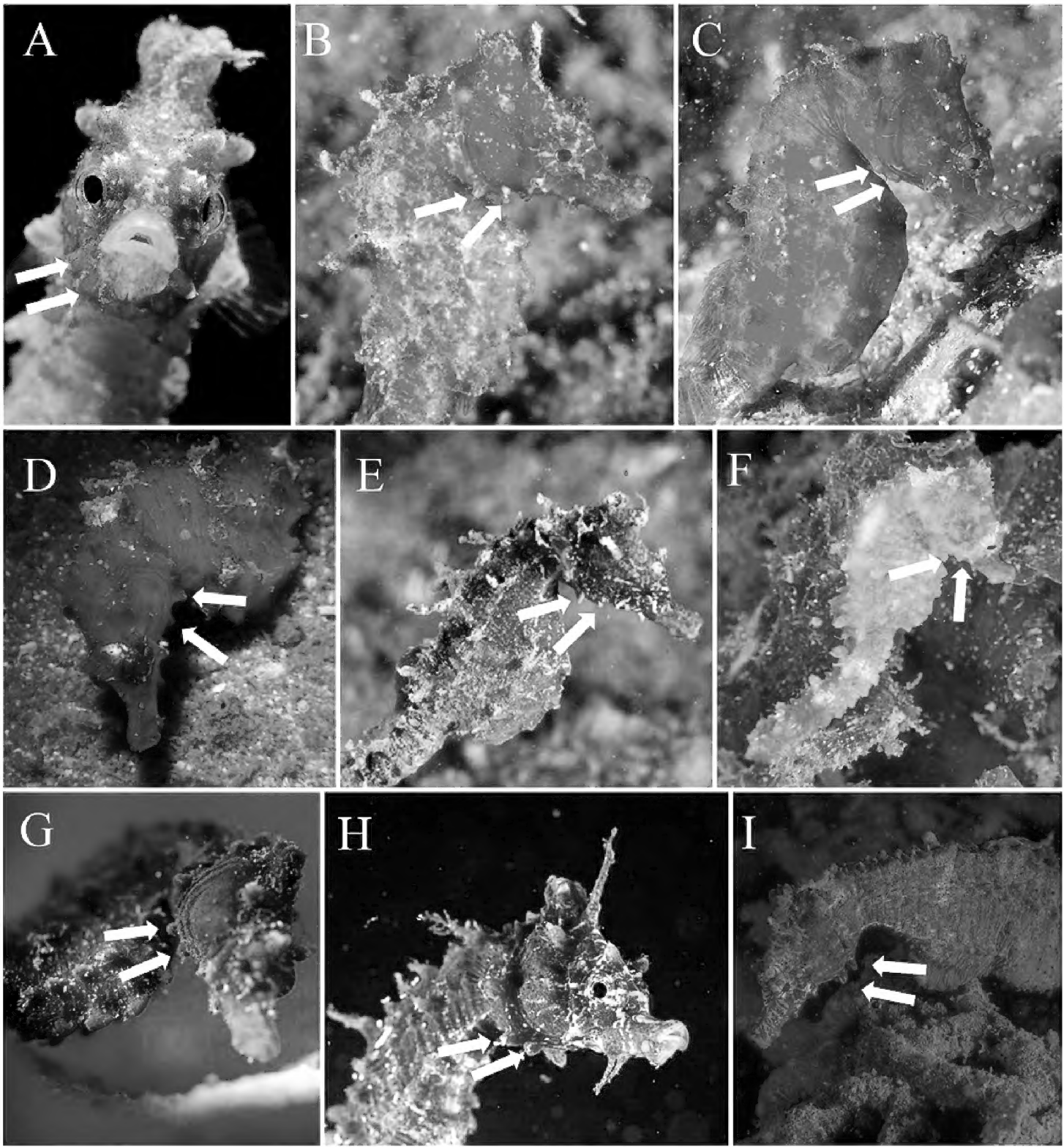

Figure 1. Photographs of Japanese seahorse *Hippocampus mohnikei* from 2013 to 2020. Arrows indicated double rounded cheek spines. **A, B.** 2013. **C.** 2015. **D.** 2016. **E.** 2017. **F.** 2018. **G, H.** 2019. **I.** 2020.
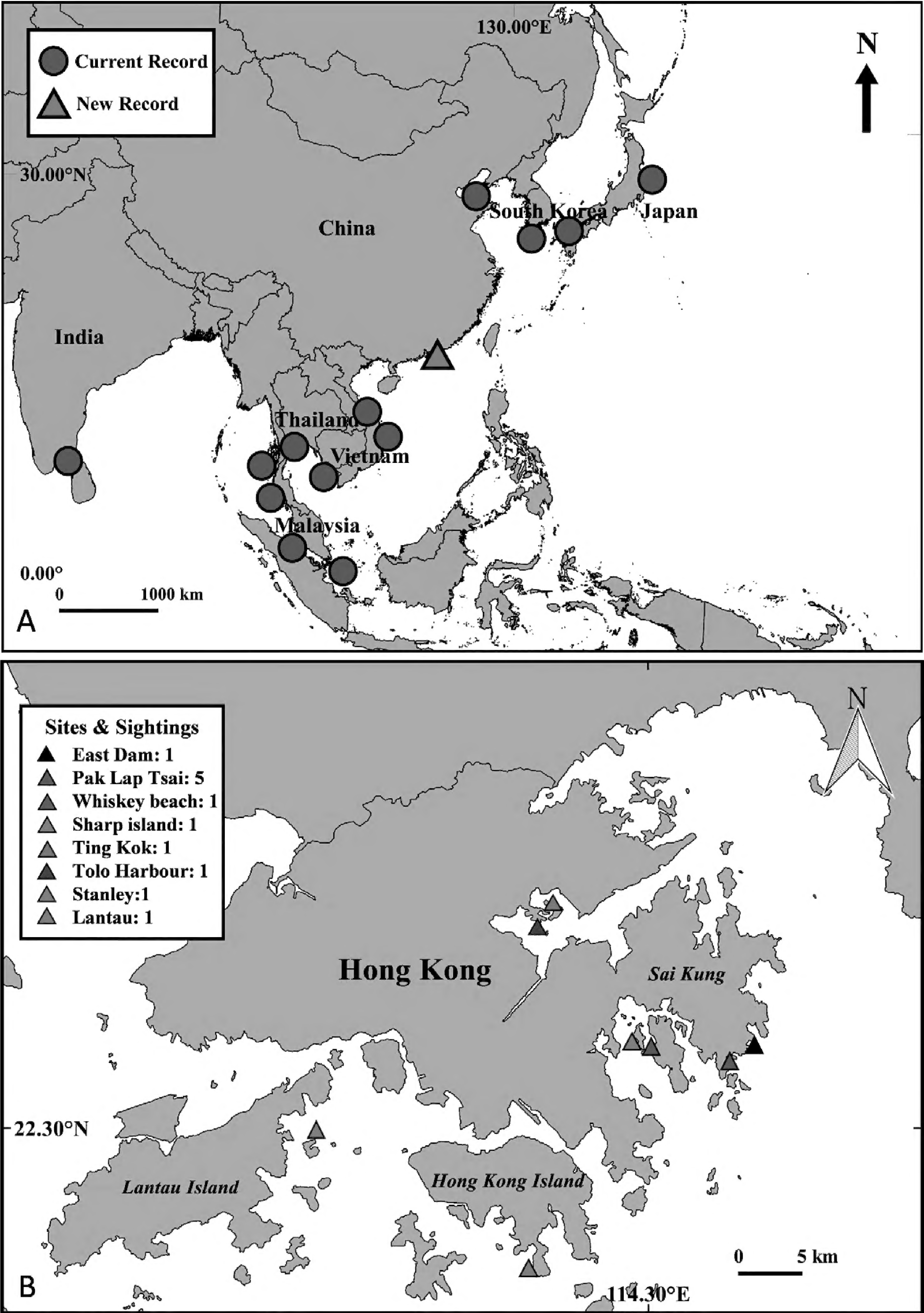
Figure 2. Map showing the locations recorded *Hippocampus mohnikei*. **A.** In Asia. **B.** In Hong Kong.

Identification. The living animal had a flattened body and long tail in proportion to body. The coronet is in the form of a ridge-like crest. It had double rounded cheek spines and a shorter snout which are the distinctive characters of *H. mohnikei*. The body color can be yellow, orange, and brown. (Fig. 1)

Remark. The details of all sightings in Hong Kong from 2013 to 2020 are listed in Table 2.

The ML tree revealed our specimens were nested in a fully support clade of *H. mohhnikei* (Fig. 3). The *p*-distance between our specimen and *H. mohhnikei* from GenBank ranged from 0.8 to 1.3% (Table 3).

Discussion

Our sighting was observed underwater in Pak Lap Tsai. The site is a small southeast-facing marine bay in the northeastern region of Hong Kong just north of Port Shelter, Sai Kung and falls within the designated Hong Kong UNESCO Geopark area. The coastline at Pak Lap Tsai is characterized by wave-cut rock structures and sea caves. All of our collected sightings largely occurred in coral communities with sandy substrates at depths of 1–20 m, which is in line with habitats described in other sightings of *H. mohnikei* in other countries (Aylesworth et al. 2016).

Combined with other local sighting sources, a total of 12 sightings of *H. mohnikei* were confirmed at eight locations in Hong Kong (Table 2). Five of the 12 sightings were in Pak Lap Tsai. There have been regular sightings of *H. mohnikei* in Hong Kong waters since 2013, with three notable sightings in 2019 alone. With the support of these continuous sightings in Hong Kong waters, it is highly possible that a population of *H. mohnikei* has been established in Hong Kong. The city now hosts four out of six (66%) seahorse species recorded in China and further affirms the region's status as a marine biodiversity hotspot for the South China Sea and China. It is likely that the *H. mohnikei* population is more widespread in Hong Kong in other areas with similar benthic habitats, but underwater surveying effort is often confined to already established or well-known dive sites. Not only is this new record important to local conservation and management, but the confirmation of a population in Hong Kong on a larger scale adds to the knowledge of the species' biogeography. Prior to these sightings in Hong Kong, the distribution of *H. mohnikei* suggested a gap between the Yellow Sea/Sea of Japan and Indo-Pacific regions. This population in Hong Kong essentially bridges the distributional gap for *H. mohnikei*.

Regarding the conservation of *Hippocampus* spp. in Hong Kong, there is a persistent issue of insufficient data for comprehensive ecological niche modelling research because there were few monitoring programmes for seahorse populations in Hong Kong. Our results demonstrate the importance and advantage of involving citizen scientists for Data Deficient species and improving

Table 2. Summary of the sightings of *H. mohnikei* in Hong Kong waters from 2013 to 2020.

Year of sighting	Location	Depth (m)	Habitats	Stage	Gender	Fig. 1	Sources	Remarks
2013	Pak Lap Tsai	5–6	Coral community, sandy bottom	Juvenile	N/A	A	Recreational diver	
2013	Pak Lap Tsai	Not reported	Coral community, sandy bottom	Adult	Female	B	Recreational diver	
2015	Pak Lap Tsai	Not reported	Coral community, sandy bottom	Adult	Female	C	Recreational diver	
2016	Pak Lap Tsai	12	Sandy bottom	Adult	Female	D	iNaturalist (SKF Yiu)	Published in OPCFHK[#] Annual Report 2016–17 (OPCFHK 2017)
2017	Whiskey beach	Not reported	Coral community, sandy bottom	Adult	Female	E	Recreational diver	
2018	Pak Lap Tsai	5–6	Coral community, sandy bottom	Adult	Female	F	This survey	
2018	Ting Kok	3	Coral community, sandy bottom	Adult	Male		Recreational diver	
2019	Sharp island	8	Coral community, sandy bottom	Juvenile	N/A	G	Recreational diver	
2019	East Dam	5–6	Coral community, sandy bottom	Adult	Female	H	Recreational diver	
2019	Lantau	1–2	Coral community	Adult	Female		Recreational diver	
2020	Stanley	20	Soft coral bottom	Adult	Male	I	Recreational diver	
2020	Tolo Harbour	Not reported	Sandy bottom	N/A	Female		iNaturalist	

[#]Ocean Park Conservation Foundation, Hong Kong.

Table 3. Uncorrected *p*-distance among the sequence of six species and local specimen.

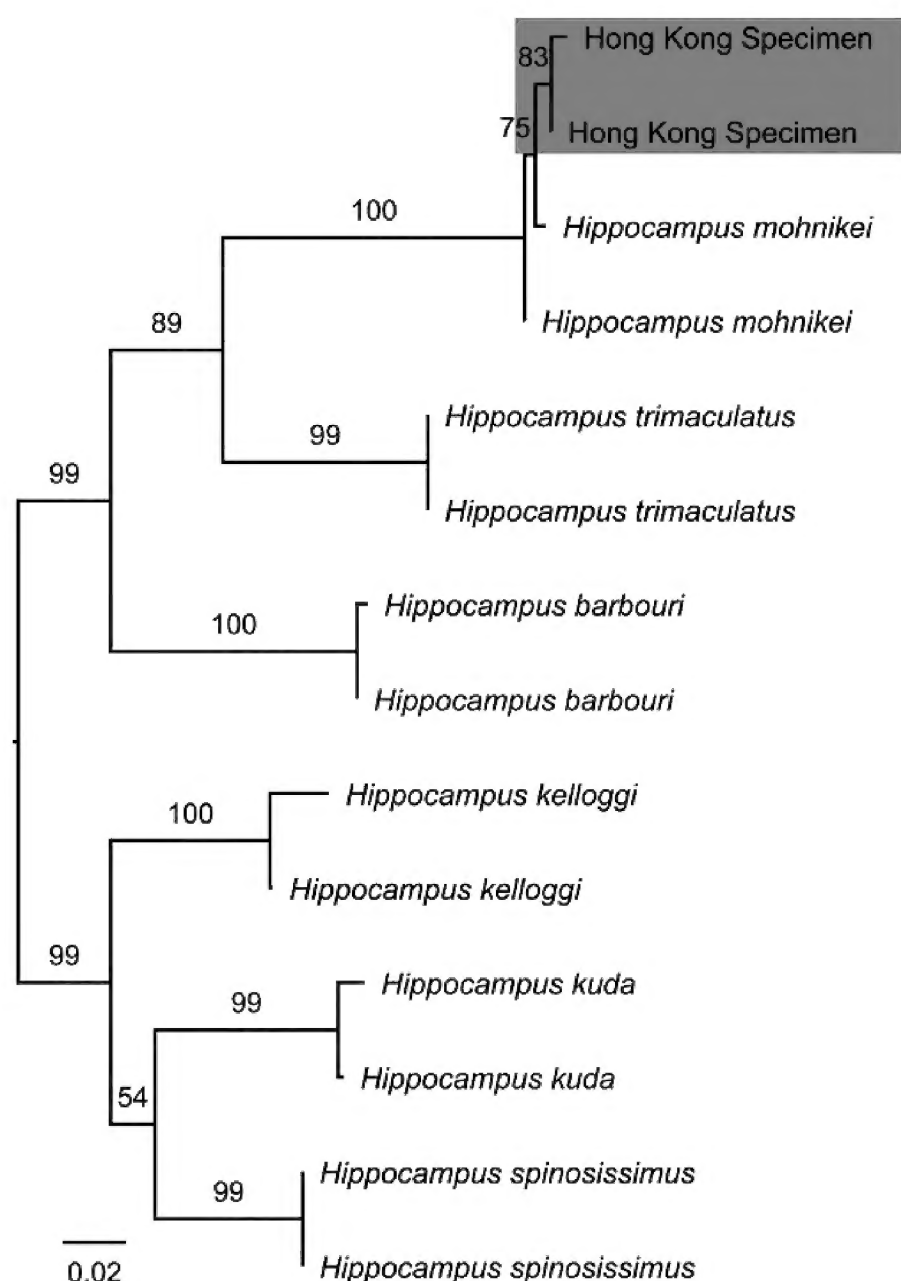|	Species	1	2	3	4	5	6	7
1	*Hippocampus barbouri*							
2	*Hippocampus kelloggi*	0.130						
3	*Hippocampus kuda*	0.124	0.101					
4	*Hippocampus mohnikei*	0.115	0.153	0.132				
5	*Hippocampus spinosissimus*	0.114	0.089	0.085	0.130			
6	*Hippocampus trimaculatus*	0.117	0.135	0.122	0.106	0.119		
7	Hong Kong specimen 1	0.120	0.148	0.130	0.013	0.128	0.109	
8	Hong Kong specimen 2	0.119	0.153	0.135	0.008	0.130	0.114	0.005

Figure 3. Phylogenetic tree of the *COI* dataset constructed using the maximum-likelihood method. Bootstrap values >50 is shown on the node.

encouraging recreational divers to be citizen scientists in the collection and submission of quality photographic data to relevant organizations and institutions can be greatly helpful towards scientific research and public awareness of marine conservation.

Apart from the morphological data, molecular data is also important to supplement the analysis. Our study demonstrated how the molecular data help to confirm the identity of the specimens with morphological data. Since there might be subtle features cannot be examined easily or morphological plasticity occurred. Hence, sometime DNA sampling is needed to supplement the analysis. The protocol of Woodall et al. (2012) is ideal to collect DNA sample with minimal impact on the individual of seahorse.

In addition, one cannot discuss conservation of a marine species without integrating habitat conservation, particularly in coral communities. As discussed above, seahorses are highly vulnerable to habitat damage due to their low mobility and small home ranges, resulting in a high site fidelity (Foster and Vincent 2004). Due to the poorly known information about *H. mohnikei* particularly in Hong Kong, specific investigations (e.g., its distribution, habitat preferences, and feeding preferences) are recommended and can be initiated, starting with Pak Lap Tsai as the first study site. Apart from filling the knowledge gap, the investigations could also help us to make efficient management and conservation strategies.

Acknowledgements

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the ecological significance of new species records. In the marine management field, challenges like counting whale sightings require vigilance over long periods of time (Davies et al. 2012). A rotating group of young students for a science project might be an ideal solution. Also, when trying to accurately identify the range of invasive marine species, volunteers can successfully cover the extensive spatial scales needed (Thiel et al. 2014). Instead of using limited university resources to send laboratory members into the field for weeks at a time, trained teachers and their schoolchildren could cover many times and more territory due to sheer numbers. To enhance the sighting of rare species underwater, the frequency of diving needs to be increased. Before this study, *H. mohnikei* had been sighted as early as 2013 by recreational divers. Most of our sightings collected were from recreational divers, which helped to support the possibility of an established population of *H. mohnikei* in Hong Kong and bridge the distributional gap in Asia. Moreover, iNaturalist is a good platform that anyone can upload the sightings with information to allow researchers to verify and access the data. Moreover, many research studies are limited in terms of their spatial and temporal scales and increasing data transparency with citizen scientists can aid scientists in detecting novel occurrences or changes in ecosystems (Beaubien and Hamman 2011; Kolok et al. 2011; Worthington et al. 2011; Bonney et al. 2014). Therefore, enabling and

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